

Product Information

Anti-phospho-Retinoblastoma (Rb) [pSer²⁴⁹/pThr²⁵²] Developed in Rabbit, Affinity Isolated Antibody

Product Number **R 3403**

Product Description

Anti-phospho-Retinoblastoma (Rb) [pSer²⁴⁹/pThr²⁵²] is developed in rabbit using a synthetic phosphorylated peptide derived from the region of human Rb that contains serine 249 and threonine 252 as immunogen (based on Swiss Protein database, accession number P06400). The sequence is conserved in human, mouse and rat (100% homology). The antiserum is preadsorbed to remove any reactivity toward non-phosphorylated Rb protein, and affinity isolated, using epitope-specific affinity chromatography.

The antibody detects human Rb protein. Mouse and rat have not been tested, however are expected to cross react. The antibody has been used in immunoblotting applications.¹

Retinoblastoma protein (Rb), the tumor suppressor product of the retinoblastoma susceptibility gene, is a 110 kDa protein that functions as a negative regulator of the cell cycle. Rb halts inappropriate proliferation by arresting cell in the G₁ phase of the cell cycle. At the transcriptional level, Rb protein exerts its growth suppressive function by binding to transcription factors including E2F-1, PU.1, ATF-2, UBF, Elf-1, and c-Abl.²⁻³

Loss of Rb function leads to uncontrolled cell growth and tumor development and is found in all retinoblastomas and in a variety of other human malignancies including cancers of breast, lung, colon, prostate, osteosarcomas, soft tissue sarcomas, and leukemia. The ability of Rb protein to alter transcription is regulated by phosphorylation, which is catalyzed by the cyclin-dependent protein kinases (cdks). Rb contains at least 16 consensus sequences for cdk phosphorylation, but the significance of all of these sites is unclear. The dephosphorylation of the Rb protein returns Rb to its active, growth suppressive state.⁴⁻⁶

Phosphorylation of serine 249 and threonine 252 is catalyzed by Cyclin cdk4. Cdk4 becomes active prior to cdk2 during G₁ cell cycle phase, and recent evidence suggests that phosphorylation of Rb by cdk4 may be required for its subsequent phosphorylation by cdk2. It has been shown that cdk4 phosphorylation inhibits HDAC binding, which is involved in DNA acetylation and active repression by Rb.⁷⁻⁹

Reagent

Anti-phospho-Rb (pSer²⁴⁹/pThr²⁵²) is provided in phosphate buffer, pH 7.4 containing 1 mg/ml BSA (protease and IgG-free) and 0.05% sodium azide.

Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

Store at -70 °C. Upon initial thawing freeze the solution in working aliquots for extended storage. Avoid repeated freezing and thawing to prevent denaturing the antibody. Do not store in frost-free freezers. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 12 months when stored appropriately.

Product Profile

The supplied reagent is sufficient for 10 blots.

A recommended working concentration of 0.25 to 1.0 µg/ml is determined by immunoblotting using Jurkat cells in high growth phase.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

Results

Peptide Competition

The specificity of this phosphorylation site specific antibody, was demonstrated by peptide competition experiment using control peptides, which have the same sequence lengths as the immunogen used to raise the antibody and the corresponding non-phosphorylated peptide.

1. Extracts prepared from Jurkat cells in high growth phase were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF.

2. Membranes were pre-incubated with following peptides:
 - Lane 1 no peptide
 - Lane 2 a generic peptide containing serine
 - Lane 3 a generic peptide containing threonine
 - Lane 4 the non-phosphorylated peptide corresponding to the immunogen
 - Lane 5 immunogen
3. Subsequently membranes were incubated with 0.50 $\mu\text{g/mL}$ of Anti-phospho-Rb [pSer²⁴⁹/pThr²⁵²].
4. After washing, membranes were incubated with goat F(ab')₂ anti-rabbit IgG alkaline phosphatase and bands were detected

The data in Figure 1 show that only the immunogen peptide Rb [pSer²⁴⁹/pThr²⁵²] blocks the antibody signal, thereby demonstrating the specificity of the antibody.

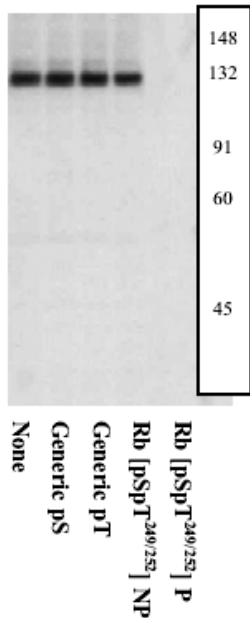


Figure 1 Peptide Competition

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